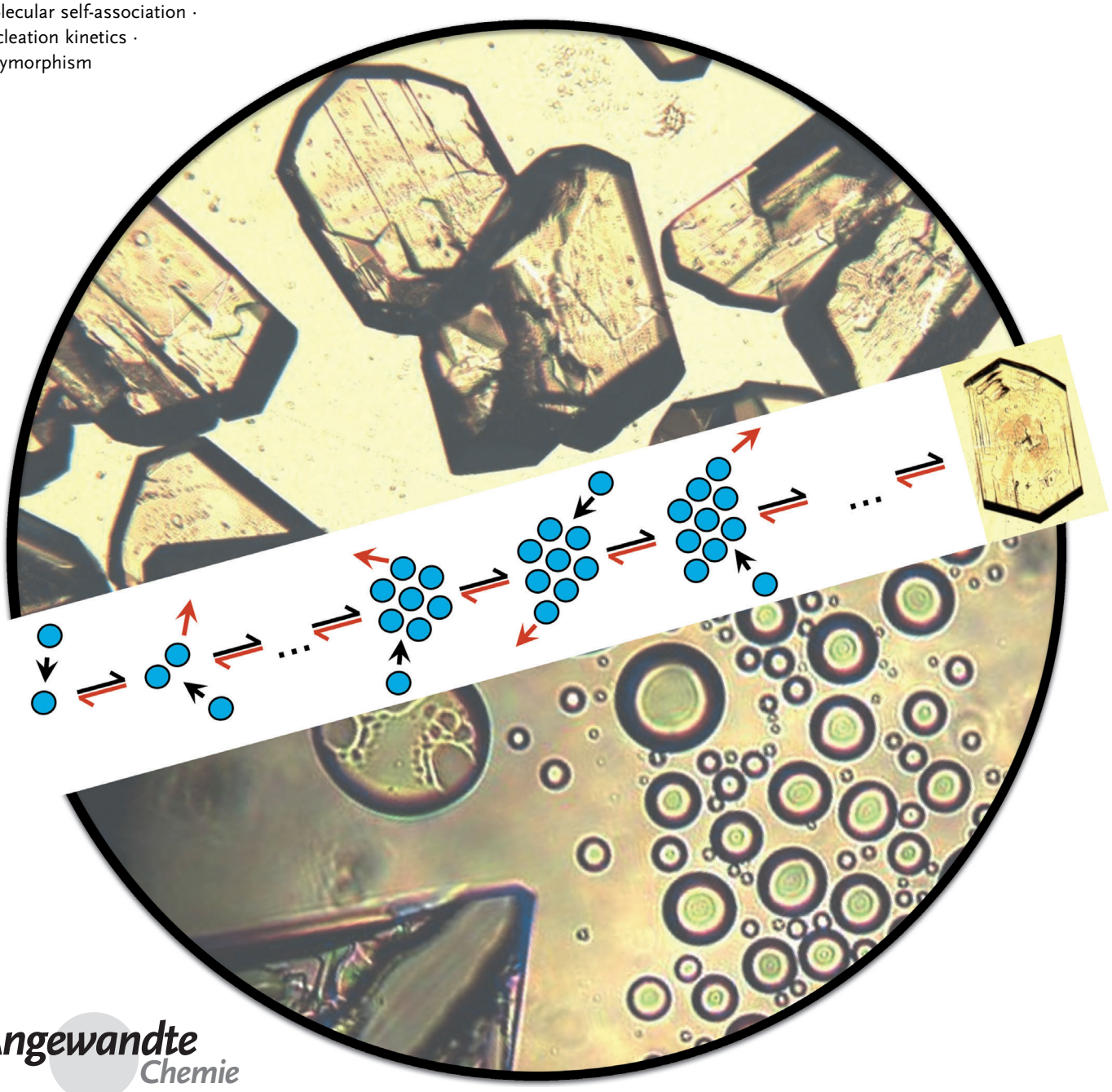
DOI: 10.1002/anie.201204824

Mechanisms of Crystallization

Nucleation of Organic Crystals—A Molecular Perspective

Roger J. Davey, Sven L. M. Schroeder, and Joop H. ter Horst*

Keywords:
crystal nucleation · crystallization processes · molecular self-association · nucleation kinetics · polymorphism



Angewandte International Edition Chemie

The outcome of synthetic procedures for crystalline organic materials strongly depends on the first steps along the molecular self-assembly pathway, a process we know as crystal nucleation. New experimental techniques and computational methodologies have spurred significant interest in understanding the detailed molecular mechanisms by which nuclei form and develop into macroscopic crystals. Although classical nucleation theory (CNT) has served well in describing the kinetics of the processes involved, new proposed nucleation mechanisms are additionally concerned with the evolution of structure and the competing nature of crystallization in polymorphic systems. In this Review, we explore the extent to which CNT and nucleation rate measurements can yield molecular-scale information on this process and summarize current knowledge relating to molecular self-assembly in nucleating systems.

From the Contents

1. Introduction

The process of phase transformation and the resulting creation of crystalline materials from liquid-phase precursors are central to the science and process engineering of materials in their broadest sense.[1] For example, in the context of materials chemistry, the ability to control with precision the process of molecular assembly from solution will be crucial for the use of structure prediction[2] and the future development of molecular-scale process design.[3] In crystal engineering, understanding and control of the key intermolecular interactions and synthons involved in the early stages of molecular self-assembly associated with nucleation are of fundamental importance for the creation of polymorphic, solvated, salt, or cocrystal solid forms with the desired physical properties.[4] Increasing interest in the molecular mechanisms of nucleation is reflected in a number of key reviews published over the last decade. For example, Davey et al.[5] reviewed links between nucleation and solution chemistry, whereas Weissbuch et al.[6] considered the stereochemical control of nucleation by the use of additives and monolayers. On the other hand, Vekilov[7] as well as Gebauer and Cölfen[8] explored mechanisms of "nonclassical" nucleation. Vekilov detailed a two-step process in which crystalline order is preceded by the separation of a dense, disordered liquid phase, as often observed in protein crystallization,[9] whereas Gebauer and Cölfen reviewed evidence for "prenucleation clusters" seen predominantly in inorganic systems. Anwar and Zahn[10] reviewed computational approaches used to elucidate the molecular nature of nucleation and pointed to difficulties inherent in the small simulation scales accessible as well as the inappropriate application of bulk thermodynamics to clusters of nanoscale dimensions.

Two mechanistic schools of thought can be identified in the area of current crystal nucleation research as summarized in Figure 1, which shows the corresponding "structural" models used to describe the dynamics of cluster formation during the nucleation process. First, there is the school of classical nucleation theory (CNT; Figure 1, top route),[11] which considers that, in a supersaturated solution, concomitant density and order fluctuations lead to the formation of clusters within which the molecular packing reflects all possible polymorphs of the solute:[6] crystal nuclei have the same structure as a mature crystal. On the other hand, "nonclassical" crystal nucleation pathways, such as a two-step mechanism (Figure 1, lower route),[7] have been identified. In this two-step mechanism, crystalline order is preceded by the separation of a dense, disordered liquid phase, and fluctua-

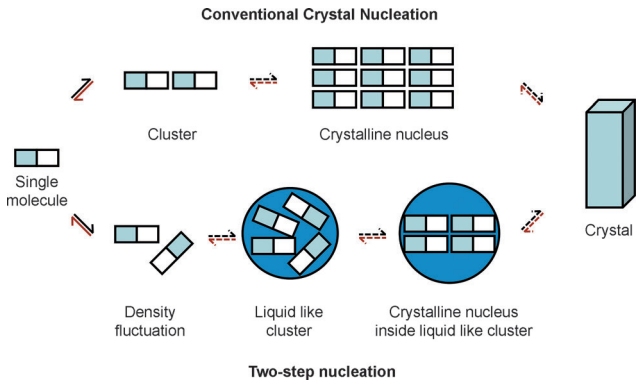


Figure 1. The two alternative structural models currently used for the dynamics of cluster formation during crystal nucleation from supersaturated solutions. Top: Density fluctuations are concomitant with fluctuations in the order parameter, so that molecular packing within clusters reflects the possible polymorphs of the solute. Bottom: Fluctuations in density are disconnected from those in order, so that the initial clusters that form are liquidlike, and crystalline order appears later on.

[*] Prof. J. H. ter Horst
Intensified Reaction and Separation Systems
Process and Energy Department, Delft University of Technology
Delft (The Netherlands)
Prof. R. J. Davey, Prof. S. L. M. Schroeder
School of Chemical Engineering and Analytical Sciences
University of Manchester, The Mill, Sackville Street
Manchester M13 9PL (UK)
E-mail: roger.davey@manchester.ac.uk
Prof. S. L. M. Schroeder
School of Chemistry, University of Manchester
Brunswick Street, Manchester M13 9PL (UK)

tions in density are disconnected from fluctuations in order: initial clusters are liquid-like, and crystalline order appears over time.

Over approximately the same period as that covered by the abovementioned reviews, there have been important advances in nucleation research as a result of the application of advanced analytical techniques, measurement methods, and molecular simulations. In situ FTIR, Raman, and UV/Vis spectroscopic techniques have given routine access to the study of solution chemistry in concentrated and supersaturated liquids, and the use of NMR spectroscopy, synchrotron-based X-ray spectroscopy, and neutron scattering have also been explored. A number of techniques for measuring crystal nucleation rates have also become available. Some are based on the supersaturation double-pulse technique[12] for the direct counting of crystals, whereas others make use of developments in microfluidics[13] and high-throughput experimentation.[14] The combined development of high-throughput experimental methods and the statistical analysis of measured induction times provides a relatively simple experimental tool with which to explore crystal nucleation kinetics.[15]

Given these advances, there is an expectation that more and more nucleation rate data for the crystal nucleation of small organic compounds from solution and information on the associated solution chemistry will become available in the near future, and that our understanding of these processes will be improved through molecular simulation. However, the interpretation of such data in terms of molecular kinetics and the structure of the crystal nucleus is still in its infancy. Our objectives for this Review are threefold: First, we reprise briefly the kinetic theory of nucleation with a focus on the limits of the molecular-scale information that may be revealed from measurement data. Second, we review alternative means by which molecular-scale insight has been gained. These approaches include X-ray spectroscopy, X-ray crystallography, molecular simulation, and studies of solution chemistry. Finally, we look to the future, when a range of methodologies that connect the thermodynamic processes of molecular assembly to cluster formation and nucleation kinetics will be needed to explore fully the molecular processes involved in nucleation and to reveal the molecular-scale nature of the transition state.

2. The Crystal Nucleation Rate

2.1. Classical Nucleation Theory

The model of a first-order phase transition involving crystal nucleation from a supersaturated phase, referred to as classical nucleation theory (CNT),[11] was developed originally by Volmer and has been continually refined over the decades.[16] The crystal nucleation rate J is the number of crystalline particles that form from a supersaturated solution per unit of volume and time. The supersaturation ratio S is a measure of the distance from equilibrium experienced by the supersaturated system. According to CNT, the crystal nucleation rate can generally be expressed by Equation (1),[17]

$$J = A \exp\left(-\frac{B}{\ln^2 S}\right) \tag{1}$$

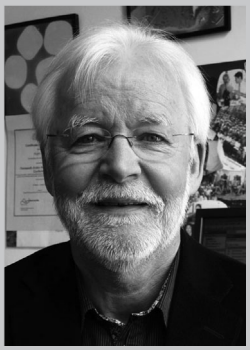
in which A and B are usually considered to be constants, and the exponent $B/\ln^2 S = W/kT$ is the dimensionless nucleation work, that is, the dimensionless energy barrier for nucleation.

It can be seen that the nucleation rate J responds nonlinearly to the supersaturation ratio S: small changes in supersaturation can induce changes of several orders of magnitude in the nucleation rate. Since we are concerned with the molecular processes that determine crystal nucleation rates, we review briefly herein how they are embedded within the pre-exponential factor A and the thermodynamic factor B. A full description of CNT can be found in a book by Kashchiev,[16] whereas a more recent account by Black[18] utilizes thermodynamic concepts to develop more practical, measurement-based applications.

2.1.1. The Thermodynamics of Crystal Nucleation

For a cluster containing n building units, the gain in bulk free energy ($-n\Delta\mu$) due to supersaturation is counterbalanced by the total interfacial energy, $c(vn)^{2/3}\gamma$, due to the presence of the nucleus–solution interface with a specific interfacial energy γ and a surface area $a = c(vn)^{2/3}$, in which c is a shape factor, and v is the molecular volume in the crystalline phase. In comparison with the bulk free energy, the total interfacial energy is relatively large for small clusters, and its contribution dominates at small cluster sizes. At relatively large cluster sizes, the bulk free energy, which is

Joop H. ter Horst studied chemical engineering at the University of Twente. As a PhD student at the Delft University of Technology (TU Delft), he investigated crystallization processes by a combination of experimental studies and molecular simulations. After a postdoctoral position, he became assistant professor at the TU Delft, where his research is focused on application-oriented fundamental aspects of crystallization processes, in particular, the elucidation of crystal nucleation and growth to enable the predictable and controlled synthesis of organic materials.



Roger Davey is Professor of Molecular Engineering in the School of Chemical Engineering and Analytical Science at the University of Manchester. He is well-known for his work on crystal polymorphism, crystal growth, and most recently solution chemistry and its relationship to crystal nucleation. His research has always had a close connection to the practical world of crystallization and formulation in the pharmaceutical industry. He is a Fellow of both the Royal Society of Chemistry and the Academy of Pharmaceutical Sciences.

related to cluster volume, becomes dominant. Thus, there is a critical cluster size, the nucleus size n^* (sometimes called the "critical nucleus size"), at which the gain in bulk free energy is balanced by the loss in free interfacial energy upon an incremental increase in cluster size. The thermodynamic parameter B in Equation (1) describes the free-energy barrier for the formation of a nucleus, the nucleation work, and is defined by Equation (2).

$$B = \frac{4}{27} c^3 v^2 \left(\frac{\gamma}{kT}\right)^3 \quad (2)$$

This expression is valid for homogeneous nucleation from clear solutions. In the case of heterogeneous nucleation mediated by, for example, random dust particles or designed templates, the interfacial energy γ should be replaced by an effective interfacial energy $\gamma_{HEN} = \psi \cdot \gamma$ to generate Equation (3).

$$B_{HEN} = \frac{4}{27} c^3 v^2 \left(\frac{\gamma_{HEN}}{kT}\right)^3 \quad (3)$$

For active templates, the activity factor is in the range $0 < \psi < 1$, which reduces the value of B and hence the nucleation work.

From Equation (1), it is evident that the nucleation work results in the existence of a metastable concentration zone during solution crystallization, within which, despite supersaturation, crystal nucleation is negligible, and beyond which the supersaturated system is labile. These thermodynamic considerations explain the observed kinetic phenomenon of the delayed occurrence of crystal nucleation. The molecular-packing motif within the nucleus and the interaction between the solution and the nucleus surface influence the specific interfacial energy γ, and to this extent the parameter B contains molecular-scale information about the nucleation process. In CNT, the interfacial energy of the nucleus is assumed to equal that of an infinitely large surface in contact with the same solution.[16] For a crystalline compound with anisotropic interfacial energies, which is the usual case for molecular crystal nucleation, this assumption means that the appropriate interfacial energy is a weighted average over all facets of the nucleus surface, as highlighted in a study on aspirin by Hammond et al.:[19] by using calculated interfacial energies together with variations in crystal habit, it was shown that the effective interfacial energy can vary from 6 to 42 mJ m^{-2} in aqueous ethanol as the proportion of hydrophobic surfaces increases. Thus, the shape factor c and interfacial energy γ in Equation (3) are interdependent, which means on the one hand that unless the morphology of the nucleus is known, the value of γ cannot be calculated, and on the other that from a single experimental value of γ, the morphology cannot be inferred. This point is reinforced by the fact that all attempts to correlate γ for a range of materials focus on solubility[20–22] as the bulk variable rather than any feature of the respective crystal structures.

Sven L. M. Schroeder is Professor of Applied Physical Chemistry in the School of Chemical Engineering and Analytical Science at the University of Manchester. He holds a chemistry degree from the Freie Universität Berlin and a PhD in chemistry from the University of Cambridge. He specializes in the analysis of local chemical and physical interactions, particularly at interfaces, by X-ray spectroscopy and collaborates extensively with industry. His recent research spearheads the characterization of organic crystals, their nuclei, and their solutions by X-ray spectroscopy.

2.1.2. The Kinetics of Crystal Nucleation

Whereas the thermodynamic factor B reflects the structure of the nucleus, the pre-exponential factor A in Equation (1) describes the molecular kinetics of the nucleation process. In the Szilard–Farkas model, nucleation is assumed to be a consecutive series of attachments and detachments to form differently sized clusters of the nucleating phase in the supersaturated phase (Figure 2).[16,22] In the case of a stationary state with a constant cluster concentration, the nucleation rate can then be defined as the product of the actual nucleus concentration X^*, the frequency f^* of the attachment of building units to the nucleus, and a factor ξ[23,24] to account for the fraction of clusters of size (n^*+1) that eventually decay rather than grow out. In this way (see Figure 2), the pre-exponential factor A is ultimately defined by Equation (4).

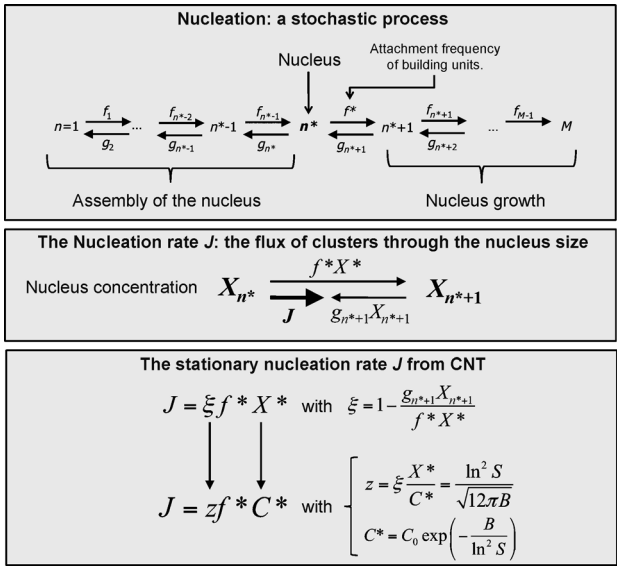


Figure 2. The nucleation rate is modeled as a flux through the size n^* of the nucleus. Ultimately, the nucleation rate is expressed as a product of the Zeldovich factor z, the frequency of attachment f^* of building units to the nucleus, and the nucleus concentration C^* that would exist in the equilibrium between the nuclei and the supersaturated solution. The Zeldovich factor accounts for the use of C^* instead of the actual nucleus concentration X^* and for the use of clusters larger than the nucleus that eventually decay rather than grow out to macroscopic size.

$$A = zf^*C_0 \quad (4)$$

In this equation, f^* is the important molecular-level kinetic contribution to the nucleation rate, since not only does it reflect how the building units attach to the nucleus, but it can also be assumed that for crystal nucleation from solution this step is the rate-limiting step.[16] This stochastic process of random attachments and detachments towards the growth or decay of a cluster with a certain size can be described well by using Monte Carlo type molecular simulations, which give simulated nucleation rates.[25,26]

During the assembly of a cluster, building units must transfer from a dissolved, solvated state to an adsorbed, partially desolvated state on the nucleus surface. If an attachment frequency controlled by interfacial transfer is assumed, the rate that building units enter into this adsorbed state is a function of the nucleus surface area A^* in contact with the solution, a diffusion coefficient D, which describes the transfer of a building unit from the vicinity of the nucleus to a position incorporated in the nucleus, and the concentration X_1 of the building unit in the solution. The attachment frequency can thus be represented by Equation (5),[16] in

$$f^* = \lambda A^* D \frac{X_1}{d} \quad (5)$$

which d has units of length and could be assumed to be the diameter, $d = (6v/\pi)^{1/3}$, of the building unit. This value can be roughly estimated from the molecular volume v.

The sticking coefficient λ reflects that there are building units in the vicinity of the nucleus that do not make the transfer to the adsorbed state. Equation (5) is valid for homogeneous nucleation ($C_0 = v^{-1}$) but also to some extent for heterogeneous nucleation onto templates ($C_0 = C_a$).[22] The diffusion coefficient, D, is usually expressed in terms of an energy barrier ($D = D_0 \exp(-E/RT)$), in which the activation energy E may be associated with partial loss of the solvent shell and/or a conformational change of the molecule upon incorporation into a cluster.[22] Effectively, this results in an exponential term included in the pre-exponential factor A in Equation (1). This state of affairs had already been identified in 1954 by Dunning and Shipman during studies on sucrose[27] in which the temperature dependence of the pre-exponential factor gave an activation barrier E of 66 kJ mol^{-1} and suggested, as expected, a large entropic penalty in the creation of the activated state of the molecule being incorporated. In the past, this energy barrier E has often been neglected because of the vast amount of work needed to measure nucleation rate as a function of both supersaturation and temperature.

Thus, if we manage to obtain the pre-exponential factor from heterogeneous nucleation rate data, we can obtain the product f^*C_0 of attachment frequency and the concentration of nucleation sites with the aid of Equation (4) and an expression for the supersaturation-dependent Zeldovich factor, z.[15] It is often stated that C_0 may be associated with heterogeneous dust particles. However, neither the concentration of such particles nor their effectiveness in promoting nucleation is known; indeed, there may be many kinds of different dust particles present. In the absence of this information, the product f^*C_0 is then the closest we can get to molecular kinetics. If we assume arbitrarily that C_0 is roughly constant in a given laboratory, then we might compare trends in f^* with respect to the solute, solvent, and temperature.

On the other hand, it would be possible to use well-defined heterogeneous designer templates with a specific size and specific interfacial energies[28–30] so that the concentration C_a of nucleation sites and the activity factor ψ are known independently. The attachment frequency f^* can then be determined, and the effect of a particular parameter, for example, the solvent, on the attachment frequency f^* can be analyzed. Alternatively, one can attempt to eliminate heterogeneous nucleation altogether. It might be possible to eliminate heterogeneous nucleation in sufficiently small volumes by the use of emulsions,[31] in microfluidic devices,[32] and perhaps also at planar liquid–liquid interfaces[33,34] and in levitated droplets.[35,36] However, the interface of the small volume may itself act as a heterogeneous surface that promotes heterogeneous nucleation.

Table 1 gives some typical measured values of A, B, and γ for a number of small molecules and the protein lysozyme. Whereas the values of γ inferred from values of B are in line with calculations, the experimentally determined values of A are much lower than best estimates. For example, for a sparingly soluble inorganic salt, Kashchiev and van Rosmalen[22] suggest a value of 10^{32} m^{-3} s^{-1} for homogeneous nucleation and values between 10^{15} and 10^{25} m^{-3} s^{-1} for heterogeneous nucleation. For the homogeneous nucleation of benzoic acid from a 2-propanol/water mixture, we estimate an A value of 10^{34} m^{-3} s^{-1} from the values $\gamma = 3$ mJ m^{-2} and $S = 1.4$.[37] According to Vekilov,[7] an A value of 10^{26} m^{-3} s^{-1} is a reasonable estimate for the homogeneous nucleation of lysozyme. It is evident from Table 1 that estimates of the pre-exponential value are always significantly in excess of the measured values. This situation was noted as early as 1957 in

Table 1: Measured values for A and B in Equation (1).

	A [m^{-3} s^{-1}]	B	γ_{eff} [mJ m^{-2}]	ψ
benzoic acid in toluene[101]	17.9×10^3	0.67	4.95	0.25
m-aminobenzoic acid in ethanol[15]	87×10^6	3.6	8.7	0.27
L-histidine in water[15]	36.3×10^3	1.1	5.1	0.22
RDX in acetone/water[38]	10^6		9.6–5.2	
lysozyme in aqueous sodium chloride solution (3%)[102]	approx. 10^8	33	0.51	
H_4EDTA in water[103]	5.7×10^{15}	330	21	
paracetamol in water[40]	$1–2.9\times10^3$	0.001–0.029	0.48–1.47	

a study on cyclotrimethylene trinitramine (the energetic organic material RDX),[38] whereby the discrepancy was thought to be connected with the large energy barrier for the attachment of molecules to the nucleus. However, a low concentration of nucleation sites could also explain these low values. A further factor which may lead to this mismatch of theory and experiment is an observed single-nucleus mechanism[39,40] in crystallizations from solution: in a stirred solution, a single nucleus is apparently formed initially and after growth to a substantial size undergoes secondary nucleation, probably by collisions with the stirrer, to form a suspension. This mechanism is best exemplified by the chiral symmetry breaking observed during the crystallization of sodium chlorate from a stirred solution,[41] whereby spontaneous nucleation can give rise to a suspension of crystals of a single chirality. This outcome shows that all crystals in the final suspension must have had a common ancestor.

In the context of the molecular processes involved in nucleation, two points can therefore be stressed. First, the single value of the interfacial energy γ obtained from a correlation of data by the use of Equation (1) is insufficient to generate a reliable view of the packing within the nucleus. We would need separate measurements of the structure of clusters in solution to answer the question: "What is the structure of the nucleus?" Second, obtained values of A will make it possible to estimate f^*C_0, but the molecular-attachment rate f^* will remain elusive except under very exceptional circumstances.

It is thus evident that although the availability of reliable methods for measuring nucleation rates may lead to an increase in kinetic data, the difficulty of using such data to gain a molecular-scale interpretation of the nucleation process remains. Anwar and Zahn[10] go further in stressing that it is becoming evident from simulations that the treatment of nuclei as tiny crystallites with bulk properties is not valid, and that the challenge for the science of crystal nucleation from solution is to establish the actual structure of the nucleus and its physical properties, in conjunction with the development of more realistic theoretical models that rely less on the traditional simplifications.

2.2. Nonclassical Nucleation

Vekilov[7] as well as Gebauer and Cölfen[8] have explored mechanisms of "nonclassical" nucleation. Vekilov detailed a two-step process in which crystalline order is preceded by the separation of a dense, disordered liquid phase (Figure 1). This two-step process was first identified in simulations.[42] Experimentally, it has been described predominantly for protein systems,[7,43] but can also be observed when small molecules crystallize.[44] Gebauer and Cölfen reviewed evidence for "prenucleation clusters" seen predominantly in inorganic systems.

Although this two-step mechanism is indeed plausible, we are concerned that there exists some confusion about it. On the one hand it seems quite possible that a nucleus may start life as an amorphous entity, which through solid–solid transitions undergoes densification and ordering to yield a critical or supercritical nucleus with the structure of the bulk crystal, as in the Lennard–Jones simulation of Anwar and Boateng[45] and the computational study of clathrate hydrates by Jacobsen et al.[46] It is also conceivable that such a cluster may form by the aggregation of thermodynamically stable prenucleation clusters.[8] On the other hand, we do not consider this process to be the same as the liquid–liquid phase separation (oiling out) which is sometimes observed when proteins and small molecules crystallize[44] and which leads to a dispersion of drops of concentrated solution within a continuous phase of less concentrated solution. This dispersed phase, which is typically visible under a microscope and has dimensions greater than a micron, arises because the supersaturated homogenous liquid phase is metastable with respect to two liquid phases, one rich and one lean in solute (a submerged liquid–liquid miscibility gap).[47] Thus, the initially single homogeneous liquid separates into two homogeneous supersaturated liquids in equilibrium, as shown schematically in Figure 3. In principle, crystal nucleation can still proceed in

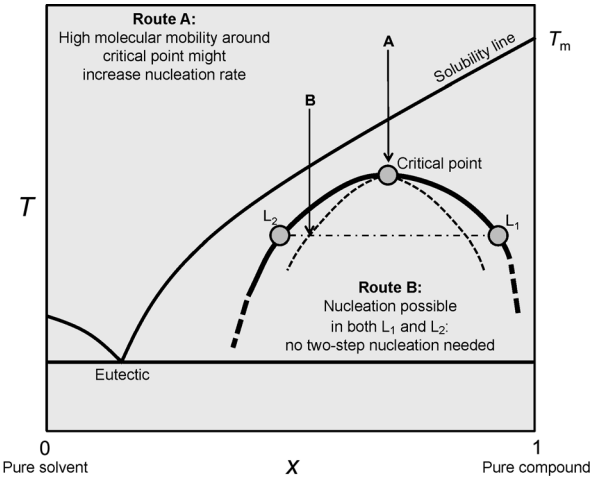


Figure 3. Binary phase diagram containing a solubility line and a submerged region of metastable liquid–liquid phase separation. Upon the cooling of a solution, the solubility line is crossed without the occurrence of nucleation. In route A, the liquid–liquid region is approached exactly at the critical point, at which the liquid separates into two liquids of equal composition. The higher molecular mobility around the critical point might result in a higher nucleation rate. In route B, the liquid–liquid region is entered, and oiling out occurs into liquid phases with compositions L_1 (relatively rich in the compound to be crystallized) and L_2 (relatively lean in the compound to be crystallized). Both liquid phases are equally supersaturated with respect to the compound to be crystallized; thus, nucleation can occur in both phases. Crystal nucleation by these routes can follow either a classical or a nonclassical mechanism.

both phases. The nucleation mechanism, however, remains the same, be it classical or nonclassical: the act of liquid–liquid phase separation has no bearing on the nucleation mechanism other than to offer two different compositional environments. Thus, liquid–liquid phase separation has nothing to do with the mechanism of nucleation: the drops of concentrated liquid phase are not prenuclei, they are another bulk phase. If the solution composition and the cooling profile are such that the liquid–liquid phase boundary is crossed at

the critical point (the point at which the liquid would split into two liquids of equal composition), then the liquid–liquid spinodal region is reached, in which molecular mobility is high and liquid–liquid phase separation proceeds spontaneously. This enhanced molecular mobility may increase the chance of crystal nuclei appearing, and hence the nucleation rate may be higher than expected.[7]

Overall, we conclude that the existence of a submerged liquid–liquid miscibility gap should not be confused with fundamental mechanisms of nucleation. Neither, as pointed out by Gebauer and Cölfen,[8] should such a two-step mechanism be confused with the existence of prenucleation clusters, since the latter are not nucleated and do not represent a separate phase but are actually thermodynamically stable associates in equilibrium with solute monomers.

In summary, CNT offers little insight into the structure of nuclei, and the more recent nonclassical alternatives are little better.

3. Insight into Molecular Processes

Since nucleation studies alone cannot provide the information needed to gain insight into the nature of the molecular processes associated with nucleation, in this section we review other techniques and theories that have been examined.

3.1. Growth or Nucleation?

Polymorphism has been studied in much detail. In particular, the way in which solvents may direct the appearance of polymorphic crystal structures is of considerable interest. A germane case is that of 2,6-dihydroxybenzoic acid, which was reported to crystallize from toluene as a hydrogen-bonded dimer and from chloroform as a catemer. A combination of UV/Vis solution spectroscopy and crystal-structure and morphology studies supported the idea that the solvent toluene favored one polymorph (solutions in toluene were rich in the dimer), whereas the more polar solvent, chloroform, inhibited dimer formation and favored the catemer structure.[48]

Of course there is danger in assuming that the macroscopic appearance of a crystal form can be linked directly to processes that occur during nucleation. In reality, the relative crystal-growth rates of available polymorphs may play a major part.[20,49] This danger is inherent to many attempts to explain the empirical "law of stages" proposed by Ostwald, as discussed by Hammond et al.[50] in relation to their study on glutamic acid clusters and highlighted in both the modeling study of Desgranges and Delhommelle[51] on the mechanism underlying polymorph selection during the crystallization of a charge-stabilized colloidal suspension and recently reported experiments on the crystallization of the α and γ polymorphs of glycine from aqueous solution.[52] In this last case, the facile appearance of the metastable dimer-based α form had previously been attributed to the existence of dimers in aqueous solution. The observation that changes in the pH value and the presence of various additives could favor the formation of the γ form was explained by a reduction in the number of dimers or the selective inhibition of α over γ nucleation.[53] Recent crystal-growth studies[52] raised doubts over this interpretation of the data: in fact, the presence of additives and higher pH values actually enhances the growth rate of the γ polymorph relative to that of the α polymorph, so that the γ form dominates the crystallization outcome. Thus, this result has nothing to do with nucleation but rather with growth.

From a theoretical point of view, nucleation cannot be inhibited by the presence of small concentrations of impurities or additives: owing to their low concentration they influence only a small part of the total volume so that nucleation takes place uninterrupted in the major part of the solution. Nucleation can be enhanced, however, if impurities or additives act as efficient heterogeneous particles. Conversely, of course, small amounts of impurities or additives can have a strong effect on crystal growth, since once they are present on the growing surface, they are hard to remove and even at very low levels block growth at the crystal surface.

3.2. X-ray Methods—The Structure of the Nucleus

The small percentage of molecules involved in clusters and the nanometer size of the clusters make the experimental elucidation of nucleus development and structure difficult. Attempts have been made to use simultaneous small-angle and wide-angle X-ray scattering (SAXS and WAXS) for the detection and structural evaluation of a nucleating phase.[54–56] In the case of 2,6-dibromo-4-nitroaniline, these data suggested the existence of an initial amorphous phase, which becomes crystalline within a few tenths of a second.[55] For several inorganic systems it has been shown that when these techniques are combined with short-range structure determination through core-level X-ray spectroscopy, further information can be obtained about local ordering phenomena around the X-ray-absorbing atoms.[56–58] Studies reported to date suggest that the first phase to appear is not crystalline for a zinc-doped molecular sieve,[56] gold-nanoparticle formation,[57] and iron oxide crystallization.[58] On the other hand, experiments in which the sensitivity of grazing incidence X-ray diffraction was used to study the behavior of molecular layers at the air–water interface[6] showed that even a few molecular layers may have the packing of a mature crystal and be influenced by the nature of the subphase and the presence of additives.

Despite these experimental limitations, some researchers have used bulk structural data derived from single-crystal and powder X-ray diffraction to infer information about the nucleus. The idea of a crystal as a supramolecular entity and the nucleus as a transition state in its formation has been invoked as a rationale for the use of such data to shed light on the packing characteristics in the nucleus. However, there is an inherent problem with this approach in that traditional crystallography deals with the macroscopic world of periodic structures and does not enable the investigation of events at the level of the nucleus. Thus, extrapolation must be viewed with caution.

For example, a study of the crystallization of *p*-azoxyanisole from its melt by the use of a combination of SAXS/WAXS, FTIR spectroscopy, and X-ray crystallography illustrated that the first phase to form from a hexagonally packed nematic liquid is not the stable crystal form but a metastable crystalline polymorph with a packing[59] related to the orientational order of the nematic phase. If this macroscopic behavior is taken as a model for nucleation in a supersaturated solution, then it becomes apparent that the nucleation pathway involves clusters in which molecules first adopt the weakly ordered nematic packing before undergoing a phase transition to a fully ordered crystal form. The preorganization due to the liquid-crystal phase may direct the structural outcome, but there is no evidence that it circumvents the nucleation process: a significant nucleation barrier still exists.

In a structural study of sodium saccharinate dihydrate, Desiraju and co-workers[60] found that the unit cell contained 64 Na^+ cations, 64 sac^- anions, and 120 water molecules and was thus unusually large and complex for such small and simple ions and molecules. Furthermore, they noted that part of the cell, a section containing six saccharinate ions together with their associated sodium ions and water, was disordered. Accordingly, again on the basis of the bulk structure as a model for nanoscale features, they argued that in this case the disorder of the unit cell, as determined from a macroscopic crystal, is a reflection of the disordered nucleus and gives a good idea of what such a transition state may look like. However, the fact that this disorder is seen at macroscopic crystal sizes means that it must have been continually built into every unit cell throughout the entire growth process of the crystal and hence has nothing to do with nucleation but rather with growth. In other cases,[61] it was argued that solvate formation resulted from a reluctance to expel solvent from a nucleus: "interrupted crystallization". This argument is flawed for the same reason and further undermined by the fact that the macroscopic formation of stable solvates is also dictated by thermodynamics.

One case in which crystallography may help is in twinning, in which a single twin plane is formed at the time of nucleation. An example is found in saccharin,[62] in which the formation of mirror twins is solvent-dependent and occurs only at the point of nucleation. Modeling revealed that, although in the known crystal structure the only hydrogen bonds are those within amide dimers, the twin plane exhibits a bonding pattern involving a three-center hydrogen bond (a new C=O···H interaction) not seen in the mature crystals and may thus truly reflect an alternative packing available only at nucleus sizes and only when the correct building unit is present in solution. This observation supports the idea that a cluster can adopt a packing that is not favorable in a mature crystal.

Overall, we note that traditional crystallographic methods have an important role to play in the interpretation of nucleation experiments, since, for example through the Cambridge Structural Database,[63] they provide vital guidance on the conformations, packing, and motifs that may be adopted by crystallizing molecules. However, the limitations of using traditional crystallographic methods to infer information regarding the structural nature of nuclei should be carefully considered.

3.3. Solution Chemistry and Molecular Self-Association

An alternative to the view of nucleation as a nanoscale version of macroscopic solid-state chemistry is to consider it as a "scaled-up" form of solution chemistry. In solutions, whether super- or undersaturated, intermolecular interactions occur between the solvent and the solute and are usually quantified thermodynamically through the measurement of association constants.[64] Although the nature of molecular interactions and packing within multimolecular nuclei is unknown the binding in these associates formed by thermodynamically driven processes is amenable to study. The composition, size, and intermolecular interactions of these associates will be functions of the solvent, temperature, and solution composition, and these associates may be considered the building units from which clusters are created and through which they grow to nucleus size and beyond. They might be single (solvated) molecules, self-associated groups of molecules (dimers or trimers), or larger prenucleation clusters. This last possibility offers a direct link to the work of Gebauer and Cölfen.[8] Indeed, in a series of studies on solutions of citric acid monohydrate, Ohgaki and co-workers[65–67] identified solute clusters of 20 nm in size in both super- and undersaturated solutions as early as 1991 and found that this size scale correlated with the crystallite size within the resulting macroscopic crystals. In 1992, Ginde and Myerson reanalyzed earlier data on concentration gradients in columns of supersaturated aqueous solutions of glycine, citric acid, and urea and suggested the presence of clusters in the size range 1–3 nm.[68]

More recently,[69,70] a similar correspondence was found in the case of mesoscopic D,L-alanine crystals, which grow from solutions containing aggregates of 20–50 nm in size to yield crystals with a crystallite size of 50 nm. As yet, the precise relationship between such solute associates (clusters, aggregates) and the nucleus is uncertain; however, it is clear that at least at the dimer level the nature of the associate and its intermolecular binding may be an important factor in the crystal nucleation process, as shown schematically in Figure 4, in which two different dimers lead to two different polymorphic crystal structures.

The recent availability of in situ ATR spectroscopic probes (ATR = attenuated total reflectance) together with powerful neutron and X-ray sources and sophisticated modeling has made it possible to explore the chemistry of concentrated and supersaturated solutions. As in the case of crystallographic methods, these techniques enable bulk measurements to be made and are therefore not sensitive to the relatively few molecules that are actually bound up in nuclei; however, they can identify the character of the dominant small associates in solution and potentially offer answers to some relatively simple questions. For example, if association into dimers or trimers occurs in solution, is the same building unit also present in the observed macroscopic crystal? If so, is it reasonable to assume that nucleating

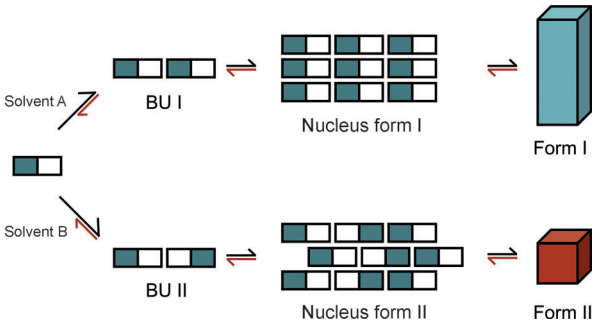


Figure 4. Self-association in different solvents might lead to the formation of different building units (BU I in solvent A, BU II in solvent B). These building units form the differently packed nuclei and thus the crystalline phases form I and form II.

clusters also contain such associates and that the CNT mechanism holds? If there is no one-to-one correlation between solution associates and crystal synthons, then it might be the clusters that act as the locus for molecular rearrangement to give the association seen in the final crystal, and this behavior might be consistent with the two-step mechanism.

Various types of solution spectroscopy, including NMR, FTIR, Raman, UV/Vis, X-ray absorption, and photoelectron spectroscopy, as well as neutron scattering have been used to gain information about solute–solute and solute–solvent associates in concentrated solutions. UV/Vis spectroscopy yields information with respect to solute dimerization versus higher-order aggregation, as demonstrated, for example, for the case of imidazole solutions.[71] The ability of core-level spectroscopic methods to probe local chemical interactions even more incisively, especially in the presence of hydrogen bonding and protonation,[72–75] has been explored in studies of imidazole solutions with X-ray photoelectron spectroscopy.[76–78] The detected chemical shifts of atomic core-level binding energies can be correlated with structural models through fundamental computational studies of clusters for the detection of local solute–solvent bonding as well as longer-range solvent structuring, coordination, and polarization.[76–78] NMR spectroscopy yields not only association constants,[79] but when combined with molecular modeling also enables visualization of the associate involved, which can be compared with synthons present in a mature crystal.[80] FTIR spectroscopy can be used to define the nature of the interactions responsible, particularly if hydrogen bonding is involved.[81] Neutron scattering with modeling by the empirical potential structure refinement (EPSR) method provides the fullest description of solution structure[82] and enables the simultaneous determination of solvent and solute radial-distribution functions—a kind of crystallography of the liquid state.

The first studies of relevance that we are aware of appeared between 2000 and 2005. They were based on NMR and FTIR spectroscopy as well as neutron scattering and went beyond the previously discussed studies on 2,6-dihydroxybenzoic acid.[48] Neutron-scattering studies on the formation of methane hydrate highlighted both a decrease in the order of the hydration shell around methane and the enhanced ordering of methane upon crystallization.[83] In the first solution NMR spectroscopic studies,[84] it was concluded from proton chemical shifts that *p*-acetanisidide molecules dissolved in chloroform were bound by intermolecular N–H···O=C hydrogen bonds identical to those in the crystal structure. Nuclear Overhauser effect data, however, suggested that these hydrogen-bonded units are packed into prenucleation aggregates, which are present in both under- and supersaturated solutions but which do not, at this extended scale, have the packing of the crystal structure. This result reflects much earlier NMR spectroscopic studies on supercooled 2-cyclooctylamino-5-nitropyridine melts, in which molecular clusters of between 2 and 6 molecules were found.[85] In an extension of such measurements, concentration-dependent proton-chemical-shift data were modeled to enable the visualization of solute dimers of an aromatic amide in chloroform and a sulfamerazine in acetonitrile and acetone.[79] In both cases, the data were consistent with a solution dimer that was identical to that in the crystal structure. For sulfamerazine, this result was independent of the solvent, in contrast with the results of an FTIR spectroscopic study in which the carbonyl and hydroxy stretches of tetrolic acid in a variety of solvents were used to identify the presence of carboxyl dimers in solution:[86] such tetrolic acid dimers were found in chloroform, whereas in ethanol, although the solute was hydrogen-bonded, it was not involved in dimer formation. This result matched precisely the known crystallization behavior of tetrolic acid, whereby a dimer-based polymorph crystallizes from nonpolar solvents, and a catemeric form from polar solvents. The FTIR spectroscopic data also revealed that in dioxane, tetrolic acid was most likely solvated. Indeed, crystallization yielded a previously unreported solvate form. These results reflected neatly the earlier molecular-dynamics studies of tetrolic acid solutions by Gavezzotti et al.[87] and are fully supported by a more recent in-depth modeling study by Chen and Trout.[88]

Overall, these two examples reveal the essential elements associated with the elucidation of crystal nucleation on the basis of the behavior of molecules in solution: solution-phase associates as inferred by spectroscopy may be confirmed and visualized through modeling and may then be compared to the synthons found in the relevant crystal structures. The link is clear from these early examples: a solution-phase dimer transfers its information to a cluster, which then grows into a crystal. A different solvent can change the nature of the solution-phase associate and hence kinetically favor another crystal form. The NMR and FTIR spectroscopic techniques were both extended to inosine, benzophenone, diphenylamine, the benzophenone–diphenylamine cocrystal, benzoic acid, and mandelic acid. Most recently, Kulkarni et al. demonstrated the reproducible effect of solvents on the formation of isonicotinamide (INA) polymorphs through the use of Raman and FTIR spectroscopy.[89] In solvents with strong hydrogen-bond acceptors, the dominant configuration of the INA molecules with respect to each other is that of amide–pyridine heterosynthons (head-to-tail chains). Similarly, solvents with strong hydrogen-bond donors lead to the dominancy of amide–amide homosynthons (head-to-head dimers). They concluded that this self-association in solution

controls the polymorph nucleation of INA by controlling which building unit attaches to the nucleus. By screening the self-association of INA in different solvents, they found indications for unassociated INA in chloroform. As discussed above for tetrolic acid in dioxane, a product with a previously unknown XRPD pattern was obtained upon crystallization. These examples suggest that association studies in solution should be incorporated into dedicated polymorph-discovery programmes.

Such studies have been complemented by the use of neutron scattering with isotopic substitution and EPSR.[90] Figures 5 and 6 compare the information generated through

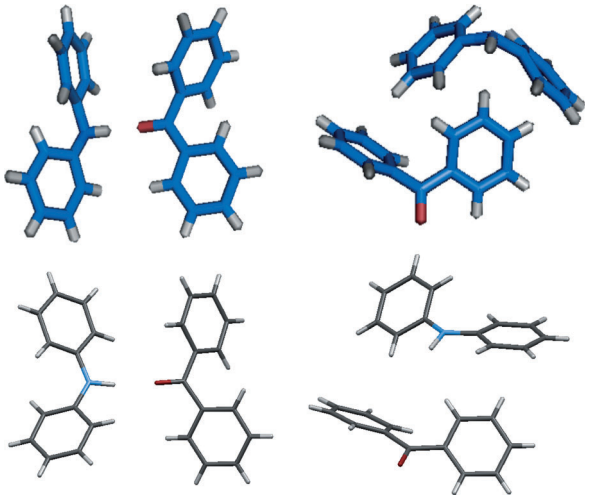


Figure 5. Association of benzophenone (BZP) and diphenylamine (DPA) as an equimolar mixture dissolved in toluene (top left) and methanol (top right), as derived by NMR spectroscopy, in comparison with the hydrogen bonding observed in the hydrogen-bonded dimer (bottom left) and face-to-edge dimer (bottom right) in the crystal structures of the 1:1 BZP/DPA cocrystal.[91] The exact dimer structure depends on the solvent used, but the crystal structure of the cocrystal can be built from either dimer.

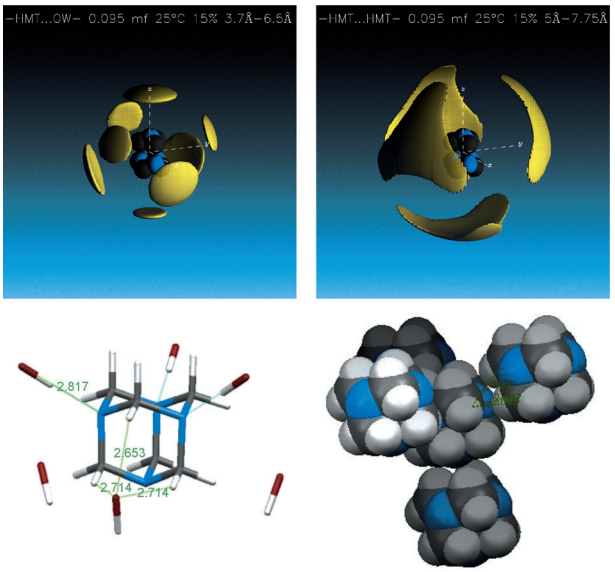


Figure 6. Results of a neutron scattering study of a 0.095 m aqueous solution of hexamethylenetetramine at 25 °C with the solution-phase spatial density functions for HMT–water correlations (top left) and HMT–HMT correlations (top right) in comparison with HMT–water coordination in the crystalline hexahydrate (bottom left) and anhydrate (bottom right).[92] Spatial density functions are three-dimensional maps showing the regions of space around a central molecule that are most likely to be occupied by the molecular centers of the neighboring molecules. For water around HMT, significant orientational order is evident, whereby HMT effectively "templates" the water through interactions with the nitrogen atoms and faces of the central HMT molecule. This behavior is reflected in the crystal structure of the hexahydrate and suggests a strong link between the structuring in solution and the appearance of a crystalline hexahydrate: the removal of two water molecules from a central HMT molecule would lead to the coordination in the crystal. For HMT–HMT interactions, the lobes above all faces of the central HMT molecule indicate the prevalence of face-to-face interactions, which mirror those seen in the crystal structure of the anhydrous phase.

the combined use of NMR spectroscopy and modeling[91] with that from neutron scattering and modeling.[92] Figure 5 compares the association of benzophenone and diphenylamine dissolved in toluene and in methanol with that in the solid state. There is clearly a solvent dependence in the interactions used to stabilize the solution dimer, but both dimers are remarkably similar to that found in the crystal. Whereas in toluene, the C=O···H–N hydrogen bond stabilizes the dimer, in methanol these polar groups are solvated, and the dimer is created through aromatic ring–ring contacts. Neutron scattering enables visualization of both solvent and solute radial-distribution functions, as seen in Figure 6 for aqueous solutions of hexamethylenetetramine (HMT).[92] In this case, the HMT–HMT correlations reflect aspects of the body-centered-cubic lattice of solid anhydrous HMT, whereas the HMT–water interactions show similarities with the crystal structure of the crystalline hexahydrate. Overall, these data remind us that before the anhydrous crystal can form, there is a need for significant desolvation to take place. This conclusion is reinforced by similar studies on supersaturated solutions of benzoic acid in methanol[93] and urea in water,[90] whereby the response of the solution to supersaturation was to enhance the extent of solvation of certain groups: in the case of urea, increased hydration of the amine groups, and for benzoic acid, enhanced solvation of the carbonyl group by methanol. The intermolecular interactions in these systems do indeed reflect those in the known crystal structures (e.g. in benzoic acid, both ring–ring and C–H···O contacts are seen), and overall the local coordination numbers are such that given the required desolvation, solute molecules could readily adopt the known crystal packing. In terms of the nucleation kinetics, this conclusion certainly supports the view that the kinetic factor A may be dominated by desolvation, which would certainly lead to much lower values than expected from CNT.

These results are summarized in Table 2, from which it is clear that in many cases there is indeed a correspondence between solution and solid-state dimers. Included in Table 2 are also examples for which the solution-phase associate does not match the synthon from the crystal structure. The chiral molecule mandelic acid provides an interesting example of

Table 2: Comparison of experimentally and computationally derived solution associates with synthons found in the respective crystal structures.

Solute (Solvent)	Technique employed	Correspondence between solution associate and crystal synthon?	Ref.
tetrolic acid (ethanol, chloroform, dioxane)	FTIR	yes (exp. and computation), polymorph and solvate	[86,88]
5-fluorouracil (nitromethane/water)	molecular modeling	yes (exp. and computation), polymorph	[104]
sulfonamides (acetone)	NMR	yes	[79]
BZP/DPA cocrystal (methanol, toluene)	NMR	yes	[91]
α-inosine (water)	NMR	yes	[80]
inosine dehydrate (water)	NMR	no	[80]
(*R*,*S*)-mandelic acid (nitromethane, acetonitrile)	FTIR	no	[81]
benzoic acid (methanol)	neutron scattering	no	[93]
3-azabicyclo-[3.3.1]nonane-2,4-dione	FTIR	yes, monomers in solution, catemer in the solid.	[94]
benzophenone (methanol, toluene)	NMR	yes	[91]
diphenylamine (methanol, toluene)	NMR	yes	[91]
p-acetanisidide (chloroform)	NMR	yes	[84]
isonicotinamide (methanol, nitromethane)	FTIR, Raman	yes	[89]
carbamazepine (methanol, chloroform)	NMR	yes	[105,106]

this situation. The crystallization of mandelic acid from racemic solutions yields one of two polymorphs of a racemic compound. In both structures, the *R*- and *S*-configured molecules are linked through the hydroxy and carbonyl groups as a centrosymmetric dimer. In saturated solutions in a number of solvents, however, FTIR spectroscopy[81] showed no evidence for such a dimer; indeed, the spectra of the racemic solutions were identical to those of the corresponding solutions of the pure enantiomers. One implication of this result is that the dimer seen in the crystal structure (one of two possible dimers) does not exist in solution: the nuclei form from monomers, and the dimer subsequently appears along the nucleation pathway, possibly through rearrangement of the clusters. A similar conclusion was reached for 3-azabicyclo[3.3.1]nonane-2,4-dione,[94] whereby the unsuccessful search for a dimer structure was attributed to the lack of dimers in solution (as judged by FTIR spectroscopy) and the fact that the modeling of clusters showed the facile breakdown of hydrogen-bonded dimers as clusters increased in size.

Overall, it is evident that for certain solute species, such as benzoic acid in methanol, mandelic acid in nitromethane, acetonititrile, and methanol, and inosine hydrate in water, there is no one-to-one correspondence between solution species and structural synthons, and a nonclassical mechanism may be involved. On the other hand, for the remaining materials in Table 2, the direct correspondence between solute association and structural synthons suggests that the application of CNT is appropriate. Such studies are worthwhile as part of a concerted attempt to shed light on the molecular processes involved in nucleation.

4. Challenges for Future Research

Owing to the increasing complexity of industrially relevant solid products, the science of crystallization must move towards the truly rational assembly of materials with targeted properties. This requirement puts considerable demands on our future ability to control crystallization processes, in particular, the crystal nucleation process, which will require rigorous predictive capabilities based on a deeper fundamental understanding of nucleation. As we have seen, new techniques have become available to measure crystal nucleation rates, but the simple collection of more nucleation rate data will not necessarily help to improve our understanding on a molecular level. In particular, more research is needed in the following areas:

Heterogeneous particles: Generally, heterogeneous nucleation is thought to occur in crystallization processes that take place at low or moderate supersaturation. Owing to the lack of well-defined heterogeneous particles in the systems examined to date, no significant progress has been made in this area in the case of small organic compounds. A thorough understanding of heterogeneous nucleation is needed and could lead to the use of well-defined templates for the enhancement of crystal nucleation. From the CNT point of view, this research would focus on the concentration of nucleation sites and the activity factor and would lead to the validation of (new) theories with respect to heterogeneous nucleation. Consequently, further insight on the molecular level could be gained from measured kinetic data.

The building unit: There are strong indications that in many cases, self-association in solution drives systems to nucleate as specific polymorphs. Currently, this self-association can often be determined with readily available analytical techniques; it is also accessible through molecular simulations. These possibilities enable an increased understanding and the routine investigation of the relationship between self-association and the kinetic data obtained for nucleation. An understanding of this relationship would lead to considerable predictive power. From a CNT perspective, this research direction reveals the nature of the building units of the

nucleus, and the nature of the building units is related to the attachment frequency of the molecules.

The nucleus: The biggest challenge is to identify the structure of low-concentration, nanosized dynamic clusters of molecules. There are some promising experimental techniques available, but to our knowledge, no relevant results have yet been reported for small organic molecules. We need a general breakthrough in this area to lead crystal nucleation research into a new era. Molecular simulations will be an essential element of this research;[9] however, it is experimental validation that is really needed. There are good grounds for the use of both neutron and light scattering together with cryo-electron microscopy to explore further the existence of prenucleation clusters in concentrated solutions of organic molecules—the existing reports for citric acid, *p*-acetanisidide and D,L-alanine suggest that this area may be fruitful territory. Dielectric spectroscopy is an emerging technique that reveals information about molecular movement during crystallization from the amorphous state,[95,96] whereas spectroscopic methods with soft X-rays, such as XPS and NEXAFS, have been shown to be sensitive to bond lengths, coordination numbers, and the geometric arrangement of coordinating species, as well as the oxidation and charge state of solute species. XPS studies of core-level binding energies associated with hydrogen bonding and protonation have already been carried out[72–78,97] and together with previous studies of core-level shifts caused by van der Waals and dipole interactions[98–100] have prepared the ground for more detailed structural studies of solute–solvent interactions, self-association, and cluster formation.

5. Summary and Outlook

Overall, although it is clear that in the field of nucleation much more data could be acquired that would be useful, the collection of rate data will not necessarily help our understanding on a molecular level unless well-designed heterogeneous templates are used to elucidate molecular kinetics. The combined use of "bulk" solid-state and solution-phase analytical tools can shed light on some structural aspects of the problem. From the small sample of available data it may be concluded that in some cases the solution associates can be built directly into clusters by the CNT mechanism to yield nuclei with the packing of a mature crystal. In other cases, clusters may contain associates which have yet to rearrange into stable packing arrangements and which may contain strongly bound solvent that needs to be removed before the stable packing can be adopted. Such clusters may indeed be referred to as amorphous, "liquid-like", or as "prenucleation clusters", and nucleation would proceed by a nonclassical mechanism. Ultimately, however, we need new approaches to the detection and analysis of molecular clusters at low concentrations in solution. These approaches will most likely develop from current state-of-the-art X-ray scattering and spectroscopic techniques in combination with sophisticated modeling of the properties of potential clusters.

R.J.D. and S.L.M.S. acknowledge support from the EPSRC. R.J.D. thanks P. E. Bonnett (Syngenta), S. N. Black, I. Ymèn (AstraZeneca), and G. Coquerel (University of Rouen) for helpful discussions.

Received: June 20, 2012
Published online: January 10, 2013

[1] J. Hulliger, *Angew. Chem.* **1994**, *106*, 151 – 171; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 143 – 162.
[2] W. I. Cross, N. Blagden, R. J. Davey, R. G. Pritchard, M. A. Neumann, R. J. Roberts, R. C. Rowe, *Cryst. Growth Des.* **2003**, *3*, 151 – 158.
[3] H. Freund, K. Sundmacher, *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim, **2011**.
[4] *Pharmaceutical Principles of Solid Dosage Forms* (Ed.: J. T. Carstensen), Technomic Publishing Co., **1993**.
[5] R. J. Davey, K. Allen, N. Blagden, W. I. Cross, H. F. Lieberman, M. J. Quayle, S. Righini, L. Seton, G. J. T. Tiddy, *CrystEngComm* **2002**, *4*, 257 – 264.
[6] I. Weissbuch, M. Lahav, L. Leiserowitz, *Cryst. Growth Des.* **2003**, *3*, 125 – 150.
[7] P. G. Vekilov, *Cryst. Growth Des.* **2010**, *10*, 5007 – 5019.
[8] D. Gebauer, H. Cölfen, *Nano Today* **2011**, *6*, 564 – 584.
[9] G. Tosi, S. Fermani, G. Falini, J. A. Gavira, J. M. G. Ruiz, *Cryst. Growth Des.* **2011**, *11*, 1542 – 1548.
[10] J. Anwar, D. Zahn, *Angew. Chem.* **2011**, *123*, 2042 – 2061; *Angew. Chem. Int. Ed.* **2011**, *50*, 1996 – 2013.
[11] M. Volmer, *Kinetik der Phasenbildung*, Steinkopff, Leipzig, **1939**.
[12] O. Galkin, P. G. Vekilov, *J. Phys. Chem. B* **1999**, *103*, 10965 – 10971.
[13] S. Selimović, Y. Jia, S. Fraden, *Cryst. Growth Des.* **2009**, *9*, 1806 – 1810.
[14] J. Leng, J. B. Salmon, *Lab Chip* **2009**, *9*, 24 – 34.
[15] S. Jiang, J. H. ter Horst, *Cryst. Growth Des.* **2011**, *11*, 256 – 261.
[16] D. Kashchiev, *Nucleation*: *Basic Theory with Applications*, Butterworth-Heinemann, Oxford, **2000**.
[17] J. W. Mullin, *Crystallization*, *4 ed.*, Butterworth-Heinemann, Oxford, **1997**.
[18] S. Black, *Proc. R. Soc. London Ser. A* **2007**, *463*, 2799 – 2811.
[19] R. B. Hammond, K. Pencheva, K. J. Roberts, T. Auffret, *J. Pharm. Sci.* **2007**, *96*, 1967 – 1973.
[20] S. Jiang, J. H. ter Horst, P. J. Jansens, *Cryst. Growth Des.* **2008**, *8*, 37 – 43.
[21] A. E. Nielsen, O. Söhnel, *J. Cryst. Growth* **1971**, *11*, 233 – 242.
[22] D. Kashchiev, G. M. van Rosmalen, *Cryst. Res. Technol.* **2003**, *38*, 555 – 574.
[23] I. V. Markov, *Crystal Growth for Beginners*, World Scientific, Singapore, **2003**.
[24] J. B. Zeldovich, *Acta Physicochim. URSS* **1943**, *18*, 1.
[25] M. A. Deij, J. H. ter Horst, H. Meekes, P. Jansens, E. Vlieg, *J. Phys. Chem. B* **2007**, *111*, 1523 – 1530.
[26] J. H. ter Horst, D. Kashchiev, *J. Chem. Phys.* **2003**, *119*, 2241 – 2246.
[27] W. J. Dunning, A. J. Shipman, *Proc. Agric. Industry Tenth Intern. Congr. Madrid* **1954**, 1448 – 1456.
[28] J. Urbanus, J. Laven, C. Roelands, J. H. ter Horst, D. Verdoes, P. J. Jansens, *Cryst. Growth Des.* **2009**, *9*, 2762 – 2769.
[29] J. Urbanus, C. Roelands, J. ter Horst, D. Verdoes, P. Jansens, *Food Bioprod. Process.* **2008**, *86*, 116 – 121.
[30] A. Y. Lee, I. S. Lee, S. S. Dettet, J. Boerner, A. S. Myerson, *J. Am. Chem. Soc.* **2005**, *127*, 14982 – 14983.
[31] D. Turnbull, J. C. Fisher, *J. Chem. Phys.* **1949**, *17*, 71 – 73.

[32] J. F. Edd, K. J. Humphry, D. Irimia, D. A. Weitz, M. Toner, *Lab Chip* **2009**, *9*, 1859 – 1865.
[33] R. A. W. Dryfe, *Adv. Chem. Phys.* **2009**, *141*, 153 – 215.
[34] M. L. Schlossman, A. M. Tikhonov, *Ann. Rev. Phys. Chem.* **2008**, *59*, 153 – 177.
[35] N. D. Draper, S. F. Bakhoum, A. E. Haddrell, G. R. Agnes, *J. Am. Chem. Soc.* **2007**, *129*, 11364 – 11377.
[36] S. K. Chung, E. H. Trinh, *J. Cryst. Growth* **1998**, *194*, 384 – 397.
[37] R. Montague, K. Back, personal communication, **2012**.
[38] W. J. Dunning, N. T. Notley, *Z. Elektrochem.* **1957**, *61*, 55 – 59.
[39] S. S. Kadam, H. J. M. Kramer, J. H. ter Horst, *Cryst. Growth Des.* **2011**, *11*, 1271 – 1277.
[40] S. S. Kadam, S. A. Kulkarni, R. Coloma Ribera, A. I. Stankiewicz, J. H. ter Horst, H. J. M. Kramer, *Chem. Eng. Sci.* **2012**, *72*, 10 – 19.
[41] D. K. Kondepudi, R. J. Kaufman, N. Singh, *Science* **1990**, *250*, 975 – 976.
[42] P. R. ten Wolde, D. Frenkel, *Science* **1997**, *277*, 1975 – 1978.
[43] D. Erdemir, A. Y. Lee, A. S. Myerson, *Acc. Chem. Res.* **2009**, *42*, 621 – 629.
[44] P. E. Bonnett, K. J. Carpenter, S. Dawson, R. J. Davey, *Chem. Commun.* **2003**, 698 – 699.
[45] J. Anwar, P. K. Boateng, *J. Am. Chem. Soc.* **1998**, *120*, 9600 – 9604.
[46] L. C. Jacobson, W. Hujo, V. Molinero, *J. Am. Chem. Soc.* **2010**, *132*, 11806 – 11811.
[47] J. E. Ricci in *The Phase Rule and Heterogeneous Equilibrium*, Van Nostrand, New York, **1951**.
[48] R. J. Davey, N. Blagden, S. Righini, H. Alison, M. J. Quayle, S. Fuller, *Cryst. Growth Des.* **2001**, *1*, 59 – 65.
[49] J. H. ter Horst, H. J. M. Kramer, P. J. Jansens, *Chem. Eng. Technol.* **2006**, *29*, 175 – 181.
[50] R. B. Hammond, K. Pencheva, K. J. Roberts, *J. Phys. Chem. B* **2005**, *109*, 19550 – 19552.
[51] C. Desgranges, J. Delhommelle, *J. Am. Chem. Soc.* **2006**, *128*, 15104 – 15105.
[52] R. Dowling, R. J. Davey, R. A. Curtis, G. J. Han, S. K. Poornachary, P. S. Chow, R. B. H. Tan, *Chem. Commun.* **2010**, *46*, 5924 – 5926.
[53] C. S. Towler, R. J. Davey, R. W. Lancaster, C. J. Price, *J. Am. Chem. Soc.* **2004**, *126*, 13347 – 13353.
[54] N. Pienack, W. Bensch, *Angew. Chem.* **2011**, *123*, 2062 – 2083; *Angew. Chem. Int. Ed.* **2011**, *50*, 2014 – 2034.
[55] H. G. Alison, R. J. Davey, J. Garside, M. J. Quayle, G. J. T. Tiddy, D. T. Clarke, G. R. Jones, *Phys. Chem. Chem. Phys.* **2003**, *5*, 4998 – 5000.
[56] A. M. Beale, A. M. J. van der Eerden, S. D. M. Jacques, O. Leynaud, M. G. O'Brien, F. Meneau, S. Nikitenko, W. Bras, B. M. Weckhuysen, *J. Am. Chem. Soc.* **2006**, *128*, 12386 – 12387.
[57] J. Polte, R. Kraehnert, M. Radtke, U. Reinholz, H. Riesemeier, A. F. Thünemann, F. Emmerling, *J. Phys. Conf. Ser.* **2010**, *247*, 012051.
[58] S. Calvin, E. E. Carpenter, V. Cestone, L. K. Kurihara, V. G. Harris, E. C. Brown, *Rev. Sci. Instrum.* **2005**, *76*, 016103.
[59] S. Janbon, R. J. Davey, G. Dent, *J. Phys. Chem. C* **2008**, *112*, 15771 – 15776.
[60] R. Banerjee, P. M. Bhatt, M. T. Kirchner, G. R. Desiraju, *Angew. Chem.* **2005**, *117*, 2571 – 2576; *Angew. Chem. Int. Ed.* **2005**, *44*, 2515 – 2520.
[61] R. Mondal, J. A. K. Howard, *CrystEngComm* **2005**, *7*, 462 – 464.
[62] H. F. Lieberman, L. Williams, R. J. Davey, R. G. Pritchard, *J. Am. Chem. Soc.* **1998**, *120*, 686 – 691.
[63] F. H. Allen, *Acta Crystallogr. Sect. B* **2002**, *58*, 380 – 388.
[64] C. A. Hunter, *Angew. Chem.* **2004**, *116*, 5424 – 5439; *Angew. Chem. Int. Ed.* **2004**, *43*, 5310 – 5324.
[65] K. Ohgaki, Y. Makihara, M. Morishita, M. Ueda, N. Hirokawa, *Chem. Eng. Sci.* **1991**, *46*, 3283 – 3287.
[66] K. Ohgaki, N. Hirokawa, M. Ueda, *Chem. Eng. Sci.* **1992**, *47*, 1819 – 1823.
[67] M. Ueda, N. Hirokawa, Y. Harano, M. Moritoki, K. Ohgaki, *J. Cryst. Growth* **1995**, *156*, 261 – 266.
[68] R. M. Ginde, A. S. Myerson, *J. Cryst. Growth* **1992**, *116*, 41 – 47.
[69] D. Schwahn, Y. R. Ma, H. Cölfen, *J. Phys. Chem. C* **2007**, *111*, 3224 – 3227.
[70] Y. R. Ma, H. Cölfen, M. Antonietti, *J. Phys. Chem. B* **2006**, *110*, 10822 – 10828.
[71] F. Peral, E. Gallego, *J. Mol. Struct.* **1997**, *415*, 187 – 196.
[72] J. S. Stevens, S. J. Byard, S. L. M. Schroeder, *Cryst. Growth Des.* **2010**, *10*, 1435 – 1442.
[73] J. S. Stevens, S. J. Byard, S. L. M. Schroeder, *J. Pharm. Sci.* **2010**, *99*, 4453 – 4457.
[74] J. S. Stevens, S. J. Byard, C. A. Muryn, S. L. M. Schroeder, *J. Phys. Chem. B* **2010**, *114*, 13961 – 13969.
[75] J. S. Stevens, S. J. Byard, C. C. Seaton, G. Sadiq, R. J. Davey, S. L. M. Schroeder, *Angew. Chem.* **2011**, *123*, 10090 – 10092; *Angew. Chem. Int. Ed.* **2011**, *50*, 9916 – 9918.
[76] B. Jagoda-Cwiklik, P. Slavícek, D. Nolting, B. Winter, P. Jungwirth, *J. Phys. Chem. B* **2008**, *112*, 7355 – 7358.
[77] B. Jagoda-Cwiklik, P. Slavícek, L. Cwiklik, D. Nolting, B. Winter, P. Jungwirth, *J. Phys. Chem. A* **2008**, *112*, 3499 – 3505.
[78] D. Nolting, N. Ottosson, M. Faubel, I. V. Hertel, B. Winter, *J. Am. Chem. Soc.* **2008**, *130*, 8150.
[79] A. Spitaleri, C. A. Hunter, J. F. McCabe, M. J. Packer, S. L. Cockroft, *CrystEngComm* **2004**, *6*, 489 – 493.
[80] R. A. Chiarella, A. L. Gillon, R. C. Burton, R. J. Davey, G. Sadiq, A. Auffret, M. Cioffi, C. A. Hunter, *Faraday Discuss. Chem. Soc.* **2007**, *136*, 179 – 193.
[81] R. J. Davey, G. Dent, R. K. Mughal, S. Parveen, *Cryst. Growth Des.* **2006**, *6*, 1788 – 1796.
[82] J. L. Finney, A. K. Soper, *Chem. Soc. Rev.* **1994**, *23*, 1 – 10.
[83] C. A. Koh, R. P. Wisbey, X. P. Wu, R. E. Westacott, A. K. Soper, *J. Chem. Phys.* **2000**, *113*, 6390 – 6397.
[84] A. Saito, K. Igarashi, M. Azuma, H. Ooshima, *J. Chem. Eng. Jpn.* **2002**, *35*, 1133 – 1139.
[85] R. Kind, O. Liechti, N. Korner, J. Hulliger, J. Dolinsek, R. Blinc, *Phys. Rev. B* **1992**, *45*, 7697 – 7703.
[86] S. Parveen, R. J. Davey, G. Dent, R. G. Pritchard, *Chem. Commun.* **2005**, 1531 – 1533.
[87] A. Gavezzotti, G. Filippini, J. Kroon, B. P. van Eijck, P. Klewinghaus, *Chem. Eur. J.* **1997**, *3*, 893 – 899.
[88] J. Chen, B. L. Trout, *J. Phys. Chem. B* **2008**, *112*, 7794 – 7802.
[89] S. A. Kulkarni, E. S. McGarrity, H. Meekes, J. H. ter Horst, *Chem. Commun.* **2012**, *48*, 4983 – 4985.
[90] R. C. Burton, E. S. Ferrari, R. J. Davey, J. Hopwood, M. J. Quayle, J. L. Finney, D. T. Bowron, *Cryst. Growth Des.* **2008**, *8*, 1559 – 1565.
[91] K. Chadwick, R. J. Davey, G. Dent, R. G. Pritchard, C. A. Hunter, D. Musumeci, *Cryst. Growth Des.* **2009**, *9*, 1990 – 1999.
[92] R. C. Burton, E. S. Ferrari, R. J. Davey, J. L. Finney, D. T. Bowron, *J. Phys. Chem. B* **2009**, *113*, 5967 – 5977.
[93] R. C. Burton, E. S. Ferrari, R. J. Davey, J. L. Finney, D. T. Bowron, *J. Phys. Chem. B* **2010**, *114*, 8807 – 8816.
[94] A. T. Hulme, A. Johnston, A. J. Florence, P. Fernandes, K. Shankland, C. T. Bedford, G. W. A. Welch, G. Sadiq, D. A. Haynes, W. D. S. Motherwell, D. A. Tocher, S. L. Price, *J. Am. Chem. Soc.* **2007**, *129*, 3649 – 3657.
[95] K. Elamin, J. Sjöström, H. Jansson, J. Swenson, *J. Chem. Phys.* **2012**, *136*, 104508.
[96] M. Jiménez-Ruiz, A. Sanz, A. Nogales, T. A. Ezquerra, *Rev. Sci. Instrum.* **2005**, *76*, 043901.
[97] D. Nolting, E. F. Aziz, N. Ottosson, M. Faubel, I. V. Hertel, B. Winter, *J. Am. Chem. Soc.* **2007**, *129*, 14068 – 14073.
[98] I. L. Bradeanu, N. Kosugi, R. Flesch, E. Rühl, *J. Phys. Chem. A* **2008**, *112*, 9192 – 9199.

[99] I. L. Bradeanu, R. Flesch, N. Kosugi, A. A. Pavlychev, E. Rühl, *Phys. Chem. Chem. Phys.* **2006**, *8*, 1906 – 1913.
[100] R. Flesch, N. Kosugi, I. L. Bradeanu, J. J. Neville, E. Rühl, *J. Chem. Phys.* **2004**, *121*, 8343 – 8350.
[101] S. Mensah, MSc thesis, The University of Manchester, **2011**.
[102] O. Galkin, P. G. Vekilov, *J. Cryst. Growth* **2001**, *232*, 63 – 76.
[103] C. P. M. Roelands, R. R. W. Roestenberg, J. H. ter Horst, H. J. M. Kramer, P. J. Jansens, *Cryst. Growth Des.* **2004**, *4*, 921 – 928; H_4EDTA = ethylenediaminetetraacetic acid.
[104] S. Hamad, C. Moon, C. R. A. Catlow, A. T. Hulme, S. L. Price, *J. Phys. Chem. B* **2006**, *110*, 3323 – 3329.
[105] C. A. Hunter, J. F. McCabe, A. Spitaleri, *CrystEngComm* **2012**, *14*, 7115 – 7117.
[106] C. A. Hunter, personal communication, **2012**.

